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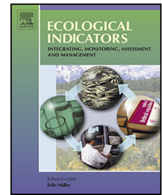

Biological indicators to assess short-term soil quality changes in forest ecosystems

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ABSTRACT

Soil performance, in terms of quality and functioning of ecosystems, has generally focused on the amount and composition of soil organic matter (SOM), but short-term SOM changes are difficult to measure. Our objective was to identify biochemical markers that are routinely used and applicable to most ecosystems as early indicators of soil quality change. A series of chemical and biochemical analyses were made in each of four seasons on soils from the Oi, Ah, BW_1, and BW_2 horizons beneath *Pinus laricio*, *Abies alba*, and *Fagus sylvatica* in Calabria Apennines, Southern Italy. Our goal was to determine not only the effect but also the relative importance of each indicator on soil quality. Microbial biomass carbon (MBC), water soluble phenols (WSP), and fluorescein diacetate hydrolase (FDA) were identified as early warning indicators of soil quality change. Seasonal changes were more pronounced for FDA activity and labile forms of SOM (WSP and MBC) than total SOM content. These three indicators reflect soil quality change due to different factors: MBC primarily reflects changes induced by vegetation, FDA displays modifications caused by climatic factors, and WSP was most sensitive to soil depth. We suggest using these biochemical indicators rather than SOM to evaluate sustainability of forest management activities.

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1. Introduction

Soil is part of the terrestrial environment and supports all terrestrial life forms. Soil quality is the result of continuous conservation and degradation processes and it represents the capacity of soil to function as a healthy living ecosystem. The need for assessing soil quality has been expanded because of new environmental constraints that affect forest ecosystem functions and plant productivity (e.g. Montreal and Helsinki processes). High quality soils are essential to maintain the integrity of terrestrial ecosystems and to recover them from disturbances, such as drought, climate change, pest infestation, pollution, and human exploitation including agriculture (Ellert et al., 1997).

The concept of soil quality includes assessment of soil properties and processes as they relate to the ability of soil to function effectively as a component of a healthy ecosystem (Weil and Magdoff, 2004). A unique balance of chemical, physical and biological components contribute to maintaining soil health. Evaluation of soil health therefore requires a broad range of indicators. Implementation of new indicators is recommended as soon as these are applicable for soil monitoring purposes. These new indicators should be based on continuous development of methods within the scientific community and they should provide more precise, detailed and integrated results, and should give an up-to date dynamic monitoring program. Implementation is recommended in parallel with existing measurements to assure the quality and comparability of the new indicator as the old indicators are phased out. The data sets of the new indicators should be used as the baseline for future monitoring activities.

For example, if soil productivity is the function of interest, a quality indicator should measure soil productivity from site to site, and detect management-induced changes within a site (Sariyildiz and Anderson, 2003). The performance of soils, in terms of quality and fertility, has been always strictly related to the amount and composition of soil organic matter (SOM) (Reeves, 1997). SOM is recognized to drive the majority of soil functions and to ensure land sustainability and restoration of degraded soils (Liang et al., 1998; Ghani et al., 2003; Hagen-Thorn et al., 2004). SOM has a myriad of interactions with other soil properties, it is a dynamic entity and its levels depend on plant factors such as productivity and litter chemistry, and on environmental factors such as temperature and water (Jenny, 1980; Burke et al., 1989). SOM amount (stock) can increase or decrease in short-time, resulting in a continuous state of flux even when stocks are at equilibrium; new inputs – via the

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process of decomposition – into and through organic matter pools of various qualities replace materials that are either transferred to other pools or mineralized. For the functioning of a soil ecosystem, this "turnover" of SOM is probably more significant than the size of SOM stocks (Six and Jastrow, 2002). It is well known that changes in SOM pools can sensitively respond to changes in plant vegetation, climate and land use in agroforestry ecosystems (Gregorich et al., 1994; Laik et al., 2009; Wang et al., 2009). However, SOM losses or gains in a short time are difficult to be measured directly because of (1) the large amount of organic matter and the low magnitude of changes; (2) the spatial variability of soils, especially of forest soils (Ghani et al., 1996; Bolinder et al., 1999; Monokrousos et al., 2004).

According to different authors (Doran and Zeiss, 2000; Knoeppa et al., 2000), soil indicators should be simple and easy to measure, should cover the largest possible situations (soil types), including temporal variations, and should be highly sensitive to environmental changes and soil management (Saviozzi et al., 2001). The indicators should be selected on the basis of their capability of assessing small variations in SOM and in soil ecosystem functioning that are linked to soil management or climatic changes, as well as on the basis of their accessibility and usefulness to producers, scientists, conservationists and policy makers (Doran and Parkin, 1996; Rezaei et al., 2006). Many soil quality indicators have been rationalized and proposed, but only a few have been tested and validated (Paz-Ferreiro and Fu, 2013). Under these premises, our goal was to identify biochemical markers to be used routinely and efficiently for different soil ecosystems, as early warning indicators of changes in soil ecosystem functioning that total SOM by itself is not able to highlight. Because of the multi-functionality of soil, it is very difficult to identify one single property as general indicator of changes in soil quality, as reported by Paz-Ferreiro and Fu (2013). Thus, the innovative objective of this research was to relate the indicators to specific factors inducing changes. We individuated vegetation, climate and soil depth as factors that could induce changes. A series of chemical and biochemical analyses were carried out in soils beneath *Pinus laricio*, *Abies alba*, and *Fagus sylvatica* in Calabria Apennines, Southern Italy, over seasons, and along soil profiles, to determine the effects and moreover the weight that the single factors have on changes in soil quality.

2. Material and methods

2.1. Study sites and soil sampling

Forest soil samples were collected in three different sites: the Regional Park of Serre (Calabria Apennines, Southern Italy), Monte Peripoli (San Lorenzo), Aspromonte, and Monte Basilicò, Aspromonte. The main characteristics of sampling sites are described in Table 1. The soils were classified according to the IUSS WRB (2006). In each site, we opened five profiles. Four different layers (horizons) were thoroughly separated from the top to the bottom of each profile on the basis of morphological differences which could be perceived by the naked eye. Every 15 days, five soil samples were taken from each horizon over a year (24 times in a year). The samples were brought to the laboratory on the same day of the collection, and kept in the refrigerator at 4 °C for up to 24 h until processing. Prior to the soil analysis, except for FDA hydrolysis and MBC, all the soil samples were air-dried, sieved (<2 mm), and visible roots were removed.

2.2. Analyses

Organic carbon was determined by dichromate oxidation (Walkley and Black, 1934). Humic substances were extracted with 0.1 M NaOH (1:10 w/v); the suspension was shaken for 16 h at room temperature, centrifuged at 5000 rpm for 30 min, and the extract was dialysed in Wisking tubes against distilled water to pH 6.0. Subsequently, the solution was filtered through a column of Amberlite IR 120 H^+. The fractionation of humic substances was carried out as follows: aliquots of extract were acidified to pH 2.0 with dilute H_2SO_4; the humic acids were precipitated and removed by centrifugation, while the fulvic acids corresponded to the supernatants (Bettany et al., 1980). The C content of humic and fulvic acids was determined by dichromate oxidation (Walkley and Black, 1934) and was expressed as percentage (%) of OM extractable in NaOH.

Microbial biomass C was determined by the chloroform fumigation-extraction procedure (Vance et al., 1987) with field moist samples (equivalent to 20 g D.W.). Soil samples were fumigated with alcohol-free $CHCl_3$ for 24 h at 24 °C. Both fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 (1:4 w/v) and filtered with Whatman's no. 42 paper. The filtered soil extracts of both fumigated and unfumigated samples were analyzed for soluble organic C using the method of Walkley and Black (1934). MBC was estimated on the basis of the differences between the organic C extracted from the fumigated soil and that from the unfumigated soil, and an extraction efficiency coefficient of 0.38 was used to convert soluble C into biomass C (Vance et al., 1987).

Microbial activity was determined by the hydrolysis of fluorescein 3,6-diacetate into fluorescein, according to Adam and Duncan (2001). Briefly, 15 ml of 60 mM potassium phosphate pH 7.6 and 0.2 ml of 1000 μg FDA ml^{-1} were added to 2 g of fresh soil. The flask was then placed in an orbital incubator at 30 °C for 20 min. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was added to terminate the reaction. The content of the flask was centrifuged at 2000 rpm for 3 min. The supernatant was filtered through Whatman no. 42. The optical density of clarified filtrates was determined at 490 nm (Shimadzu UV–Vis 2100, Japan). The enzyme activity was expressed in micrograms of fluorescein per gram of soil per hour.

Water soluble phenols were extracted with distilled water (Kaminsky and Muller, 1977, 1978). Thirty grams of dry weight samples were mixed in 200 ml distilled water and shaken at 75 rev min^{-1} for 20 h at room temperature. Solutions were filtered through Whatman's No 1 paper. All samples were extracted in triplicate. Total water-soluble phenols (monomeric and polyphenols) were determined by using the Folin-Ciocalteau reagent, following the method of Box (1983). Tannic acid was used as a standard and the concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (μg TAE g^{-1} D.W.).

In order to assess properly the variation of the biological soil properties, indices of the SOM, MBC, FDA, WSP, HC, FC and HC content, for each horizon and for the whole profile, were calculated as follows:

$$\text{Index } Y_{\text{each horizon}} = \frac{Y \times \text{Depth(cm)}}{100}$$

$$\text{Index} Y_{\text{whole profile}} = \text{the sum of the individual index}$$

$$Y = \text{SOM, MBC, FDA, WSP, HC, FC, HC}$$

2.3. Statistical analysis

A two way ANOVA was used to test the effects of plants, seasons and their interactions on SOM, MBC, FDA and WSP indices. When there were significant interactions plant and season, one-way ANOVA was used to test the effects of seasons on soil indices for each forest stand separately. Correlation analysis was used to examine relationships between SOM and the MBC, FDA and WSP variables. Treatment means were compared using Tukey's test

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